

Introduced Siberian Chipmunks (*Tamias sibiricus barberi*) Harbor More-Diverse *Borrelia burgdorferi* Sensu Lato Genospecies than Native Bank Voles (*Myodes glareolus*)[▽]

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Little attention has been given in scientific literature to how introduced species may act as a new host for native infectious agents and modify the epidemiology of a disease. In this study, we investigated whether an introduced species, the Siberian chipmunk (*Tamias sibiricus barberi*), was a potentially new reservoir host for *Borrelia burgdorferi* sensu lato, the causative agent of Lyme disease. First, we ascertained whether chipmunks were infected by all of the *B. burgdorferi* sensu lato genospecies associated with rodents and available in their source of infection, questing nymphs. Second, we determined whether the prevalence and diversity of *B. burgdorferi* sensu lato in chipmunks were similar to those of a native reservoir rodent, the bank vole (*Myodes glareolus*). Our research took place between 2006 and 2008 in a suburban French forest, where we trapped 335 chipmunks and 671 voles and collected 743 nymphs of ticks that were questing for hosts by dragging on the vegetation. We assayed for *B. burgdorferi* sensu lato with ear biopsy specimens taken from the rodents and in nymphs using PCR and restriction fragment length polymorphism (RFLP). Chipmunks were infected by the three *Borrelia* genospecies that were present in questing nymphs and that infect rodents (*B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*). In contrast, voles hosted only *B. afzelii*. Furthermore, chipmunks were more infected (35%) than voles (16%). These results may be explained by the higher exposure of chipmunks, because they harbor more ticks, or by their higher tolerance of other *B. burgdorferi* sensu lato genospecies than of *B. afzelii*. If chipmunks are competent reservoir hosts for *B. burgdorferi* sensu lato, they may spill back *B. burgdorferi* sensu lato to native communities and eventually may increase the risk of Lyme disease transmission to humans.

The introduction of pathogens infecting several hosts is one of the major causes of emerging infectious diseases (7). Many studies have documented how these introductions can affect native communities of vertebrate hosts (41). The introduction of vertebrate species also is a major driver of biodiversity change (32). Yet, how introduced species act as a new host for native infectious agents and modify disease epidemiology has received little attention (20). This issue is important because the composition of a host community has been shown to modify the transmission dynamics of infectious diseases (3).

We studied the introduction of a potentially new reservoir host for pathogenic bacteria in suburban forests, the Siberian chipmunk, *Tamias* (= *Eutamias*) *sibiricus barberi* Johnson and Jones 1955 (see reference 35). Siberian chipmunks originating from Korea have been sold in European pet shops since the 1960s. Beginning in the 1970s, they have been released intentionally into the wild. Since then, French populations have been identified in 11 suburban forests and urban parks (5).

This study allowed us to determine the consequences of the

introduction of chipmunks on the ecology of Lyme disease. This is the most prevalent vector-borne disease for humans in temperate zones of the northern hemisphere (2, 45). Lyme disease is a multihost and multipathogen disease caused by different bacterial genospecies that belong to the *Borrelia burgdorferi* sensu lato complex. In Europe, these bacteria are transmitted to humans and to reservoir hosts by hard ticks of the *Ixodes ricinus* species (37). *I. ricinus* is a generalist parasite that goes through three life stages (larva, nymph, and adult male or female). With the exception of the adult male, during each stage, the tick takes one blood meal on a vertebrate host either to moult (larvae or nymphs) or to reproduce (adult females). To encounter a host, *I. ricinus* quests on the tips of low vegetation (9). *I. ricinus* feeds on many different vertebrate species of mammals, birds, and reptiles, which can act as a reservoir host of different *B. burgdorferi* sensu lato genospecies. For example, *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. bavariensis* (previously *B. garinii* Osp A serotype 4) are associated with rodents, while *B. garinii* and *B. valaisiana* are associated with birds (12, 13). Small mammals and birds acquire their infection when a questing infected nymph attaches and feeds on them. Larvae are not considered to be infected, because the vertical transmission of *B. burgdorferi* sensu lato in ticks is very rare (19). Adult ticks only rarely feed on small mammals and birds (36).

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We pursued two objectives in this study. First, we tested whether chipmunks were infected by all of the *B. burgdorferi* sensu lato genospecies associated with rodents and which were available in their source of infection, questing nymphs. To do so, we tested whether the prevalence and diversity of *B. burgdorferi* sensu lato genospecies in chipmunks reflected that found in questing nymphs. Second, we compared the prevalence and diversity of *B. burgdorferi* sensu lato in chipmunks to those of the most abundant and known competent native reservoir rodent, the bank vole (*Myodes glareolus*) (10).

MATERIALS AND METHODS

Study site. The study was conducted 22 km southeast of Paris in the Sénart forest (3,200 ha, more than 3 million visitors per year) on the study site, La Faisanderie (14 ha, 48°39'25"N, 2°29'40"E). Siberian chipmunks have been present on the study site since the early 2000s; however, they first appeared in a northwest section of the Sénart forest in the 1970s. The forest now holds the largest population of chipmunks in France (5). In this forest, the rodent community consists mainly of Siberian chipmunks, bank voles, and wood mice (*Apodemus sylvaticus*) (J. L. Chapuis, personal observation). As few wood mice were collected during our daily trapping sessions, they were not investigated in the study.

Datum collection. As part of a broader project on the biology of introduced Siberian chipmunks that were being monitored in a capture-mark-recapture study (27, 38), infection prevalence was studied for chipmunks trapped between the months of March and October over a 3-year period (2006 to 2008). A grid made by 104 geo-localized Sherman traps baited with peanut butter and sunflower seeds was set from sunrise to sunset on an area of 14 ha (27). In 2006 and 2007, two trapping sessions, one lasting three consecutive days and the second five consecutive days, were performed during each study month (27). A 5-day-long trapping session was done every month in 2008 (39). All captured individuals were sexed. The age class (juvenile or adult) was determined by coupling the date of capture and the body weight (see reference 27). One ear biopsy specimen, used to find bacteria in blood and tissue, from each chipmunk (43) was obtained by cutting a small piece (maximum of 3 mm²) from the ear with scissors, with the sample immediately stored in 90% ethanol. Only chipmunks captured for the first time during the course of the study were analyzed.

Bank voles were caught regularly on the trapping grid (see reference 39). Up to 30 voles were sampled each month in 2006, 2007, and 2008. An ear biopsy specimen was obtained by following the same procedure described for chipmunks to detect the presence of the bacteria in blood and tissue.

Questing *I. ricinus* nymphs were sampled monthly by dragging a 1-m² cotton blanket over the vegetation (31) on two sites covering approximately 0.5 ha inside the trapping grid. Within each site, 15 units of 10 m² were randomly dragged in 2006, and 16 units were dragged in 2007 and 2008 (see reference 39).

Borrelia molecular identification. DNA from one ear biopsy specimen per chipmunk and per bank vole was extracted using the NucleoSpin tissue kit (Machery-Nagel, Düren, Germany). The presence of *B. burgdorferi* sensu lato in the extracted DNA was detected using a PCR that targets the 16S rRNA gene with 5'-ATGCACACITGGTGTTAACTA-3' (nucleotide positions 819 to 842) and 3'-GACTTATCACCGGCAGTCTTA-5' (1153 to 1173) primers (26). *B. burgdorferi* sensu lato species were identified on positive PCR products using a PCR that targets the intergenic *rrf-rrl* spacer followed by the MseI restriction pattern of products amplified with primer 1 (5'-CTGCGAGITCGCGGAGA-3') and primer 2 (5'-TCCTAGGCATTACCATA-3') (40). Nymphs were transported still alive to the laboratory, where they were analyzed for infection by *B. burgdorferi* sensu lato. A total of 30 questing nymphs collected each month from each collection site were analyzed. We proceeded using PCR on DNA directly extracted from ticks as described previously (40). Identification at the *B. burgdorferi* sensu lato species level was assessed by PCR-restriction fragment length polymorphism (RFLP) of the intergenic *rrf-rrl* spacer followed by the MseI restriction pattern. We were expecting to find a few coinfections in ticks and rodents (21, 47).

Statistical analyses. We ran a specific statistical model for each population (chipmunks, voles, and questing nymphs). For all three populations, we used a multinomial model, with the probability of being infected by every *B. burgdorferi* sensu lato species as the response variable. We separated models because each population does not host the same genospecies of *B. burgdorferi* sensu lato, and consequently, each population corresponds to a different response variable.

First, to compare the prevalence and the diversity of *B. burgdorferi* sensu lato

genospecies in questing nymphs and rodents, we selected juvenile rodents. We did not use adults because, as they may have acquired infection in previous years, their infection status would not reflect the year studied (18). Thus, we hypothesized that the rates of infection of juvenile chipmunks and juvenile bank voles were comparable with the rate of infection of questing nymphs. We estimated the probability that a nymph and young rodent would be infected by *B. burgdorferi* sensu lato and by a given genospecies of *B. burgdorferi* sensu lato (null model) by the use of generalized linear models (GLM) (29) using multinomial distribution with a logit link. The logit multinomial model is statistically equivalent to a log-linear model with Poisson distribution and a log link explaining the response variable of count conditionally to the row margin (1). We then considered explanatory factor variables typically known to influence infection prevalence in populations, i.e., year and sex (full models), of young rodents and used backward model selection. We started with fitting a model with all of the variables of interest. The least significant variable, defined as the one whose significance was below 5%, was then dropped. We continued by successively refitting reduced models, applying the same rule until all of the remaining variables were statistically significant. We did not test the year of collection for nymphs because in certain years the sampling size for some *B. burgdorferi* sensu lato genospecies was low. Differences between tick and rodent infection prevalence were compared with chi-square tests.

Second, we used the same GLM with multinomial distribution to estimate infection probability for both adult and juvenile Siberian chipmunks and bank voles (null models). We considered year, age class (adults or juveniles), sex and the interaction between age and sex as explanatory variables (full model) and used backward model selection. Differences between chipmunk and vole infection prevalence were compared with chi-square tests. All analysis programs were written with R software (R Development Core Team; 2008).

RESULTS

Study population. In total, 335 Siberian chipmunks (among which were 147 juveniles) and 671 bank voles (among which were 151 juveniles) were collected. Only nine chipmunks were coinfecting with *B. afzelii* and *B. burgdorferi* sensu stricto, and one was infected with *B. afzelii* and *B. garinii*. These 10 individuals were excluded from the statistical analysis.

In total, 893 questing nymphs were collected by dragging between 2006 and 2008. Of these, 745 were tested for *B. burgdorferi* sensu lato genospecies DNA, and 75 (10.1%) were found infected. In total, 25 (3.4%) nymphs were infected by *B. burgdorferi* sensu stricto, 21 (2.8%) by *B. afzelii*, 10 (1.3%) by *B. garinii*, 10 (1.3%) by *B. spielmanii*, 4 (0.5%) by *B. valaisiana*, and 3 (0.4%) by *B. lusitanae*, and 2 nymphs (0.3%) were coinfecting by *B. afzelii* and *B. burgdorferi* sensu stricto. These 2 coinfecting nymphs were excluded from the statistical analysis, which thus was conducted on 743 questing nymphs.

Prevalence and diversity of *B. burgdorferi* sensu lato in young rodents and questing nymphs. We found that young chipmunks hosted three of the six *B. burgdorferi* sensu lato genospecies found in questing nymphs: *B. afzelii*, *B. burgdorferi* sensu stricto, and *B. garinii*. They were overall 2.9 times more infected (29%) than questing nymphs (10%) ($\chi^2 = 39.16$, P value of <0.001) (Table 1). They also were more infected than nymphs for each of the genospecies that they harbored ($\chi^2_{B. afzelii} = 55.08$, $P < 0.001$; $\chi^2_{B. burgdorferi \text{ sensu stricto}} = 7.72$, $P = 0.005$). In contrast, young bank voles were 2.5 times less infected (4%) than questing nymphs ($\chi^2 = 4.63$, $P = 0.03$) but had a probability of being infected by *B. afzelii* similar to that of questing nymphs ($\chi^2 = 0.24$, $P = 0.62$) (Table 1). No significant factor was found when we compared the prevalence levels of *B. burgdorferi* sensu lato genospecies in young bank voles and chipmunks.

Prevalence and diversity of *B. burgdorferi* sensu lato in young and adult rodents. We found that Siberian chipmunks

TABLE 1. Estimated percentages and 95% confidence intervals for probability of being infected by *B. burgdorferi* sensu lato genospecies for questing nymphs, bank voles, and Siberian chipmunks analyzed between 2006 and 2008 in the Sénart forest^b

Question	Host species	No.	% probability (% CI) of being infected by:				
			Total <i>B. burgdorferi</i> sensu lato	<i>B. afzelii</i>	<i>B. burgdorferi</i> sensu stricto	<i>B. garinii</i>	Others ^a
Source of infection vs young rodents	Questing nymph	743	9.8 ^A ** [2.6; 16.5]	2.8 ^A [1.8; 4.4]	3.4 ^A [2.3; 5.0]	1.3 ^A [0.7; 2.5]	2.3 [1.4; 3.7]
	Chipmunk	147	29.3 ^B [13.9; 41.9]	18.4 ^B [12.5; 27.0]	8.8 ^B [5.1; 15.4]	2.0 ^A [0.6; 6.5]	
	Vole	151	4.0 ^A [1.8; 9.0]	4.0 ^A [1.8; 9.0]			
Introduced vs native (total datum set)	Chipmunk	335	35.2 ^A [25.8; 43.5]	16.1 ^A [12.3; 21.2]	17.3 [13.3; 22.5]	1.8 [0.8; 4.1]	
	Vole	671	15.8 ^B [13.0; 19.2]	15.8 ^B [13.0; 19.2]			

^a Others, other *B. burgdorferi* sensu lato genospecies (*Borrelia lusitanae*, *Borrelia valaisiana*, and *Borrelia spielmanii*).

^b Within each column and question, infection percentages that are statistically different ($P < 0.05$, χ^2 test) are shown with a different letter. CI, confidence interval.

were twice as infected (35%) as native bank voles (16%) ($\chi^2 = 47.60$, $P < 0.001$). Furthermore, they hosted two more *B. burgdorferi* sensu lato genospecies than bank voles. However, the estimated probabilities of being infected by *B. afzelii* were similar for chipmunks and voles (16%, $\chi^2 = 0.002$, $P = 0.97$) (Table 1). The probability of infection by *B. burgdorferi* sensu lato is significantly influenced by age and year for both chipmunks (age, $P = 0.017$; year, $P = 0.003$) and voles (age, $P < 0.001$; year, $P < 0.001$). Chipmunks were more infected than voles in 2007 and 2008 ($\chi^2_{2007} = 40.80$, $P < 0.001$; $\chi^2_{2008} = 46.91$, $P < 0.001$) but not in 2006 ($\chi^2_{2006} = 2.48$, $P = 0.11$) (Fig. 1). Chipmunks were less infected than voles by *B. afzelii* only in 2006 ($\chi^2 = 12.99$, $P < 0.001$) (Fig. 1). Adult rodents were more infected than juveniles ($\chi^2_{\text{chipmunk}} = 12.09$, $P < 0.001$; $\chi^2_{\text{vole}} = 7.72$, $P = 0.005$), except for chipmunks with *B. afzelii* (Fig. 2).

DISCUSSION

The mechanism by which an introduced and potential reservoir host species impacts the dynamics of a disease is unknown. We investigated whether introduced Siberian chipmunks were infected by all of the *B. burgdorferi* sensu lato

genospecies associated with rodents and compared the *B. burgdorferi* sensu lato prevalence and diversity in chipmunks with that of bank voles, a known native reservoir of Lyme disease. Data on *B. burgdorferi* sensu lato prevalence and diversity in Siberian chipmunks are scarce. No studies have investigated *B. burgdorferi* sensu lato infection in the subspecies *Tamias sibiricus barberi* in Korea. Only a few studies have reported infections in China in very low numbers of *T. sibiricus* (1 in 3 chipmunks [6] and 1 in 8 chipmunks [15]). In France, Vourc'h et al. (47) have shown that chipmunks ($n = 33$) are able to carry *B. afzelii* (9 in 11 infected individuals) and *B. burgdorferi* sensu stricto (1 in 11 infected individuals).

In our study, Siberian chipmunks were infected by *B. burgdorferi* sensu lato at a rate that was nearly twice that of their source of infection, i.e., questing nymphs, and that of the bank vole, which is the most abundant native rodent and a Lyme borreliosis reservoir. Over the 3 years of the study (2006 to 2008), their infection prevalence averaged 35%, reaching 53% in 2007, which is among the highest prevalence rates found in rodents in Europe. Such a high infection prevalence has been found only in birds (23, 46) and rodents in the United States,

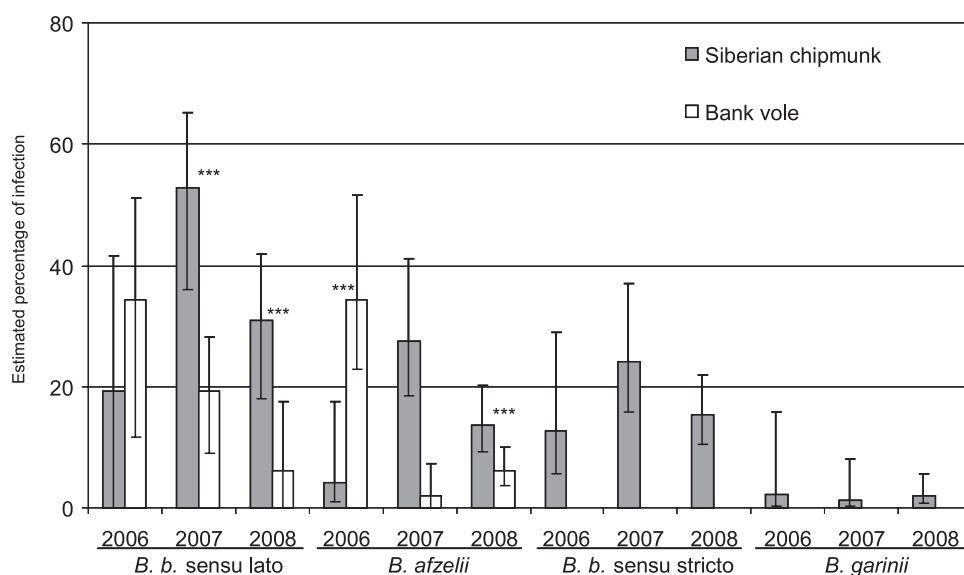


FIG. 1. Temporal variation of estimated infection percentage by *B. burgdorferi* sensu lato genospecies for Siberian chipmunks and bank voles. Significant differences ($P < 0.05$, χ^2 test) between chipmunks and bank voles within each year for *B. burgdorferi* sensu lato and *B. afzelii* are indicated with ***. Error bars represent the 95% confidence intervals.

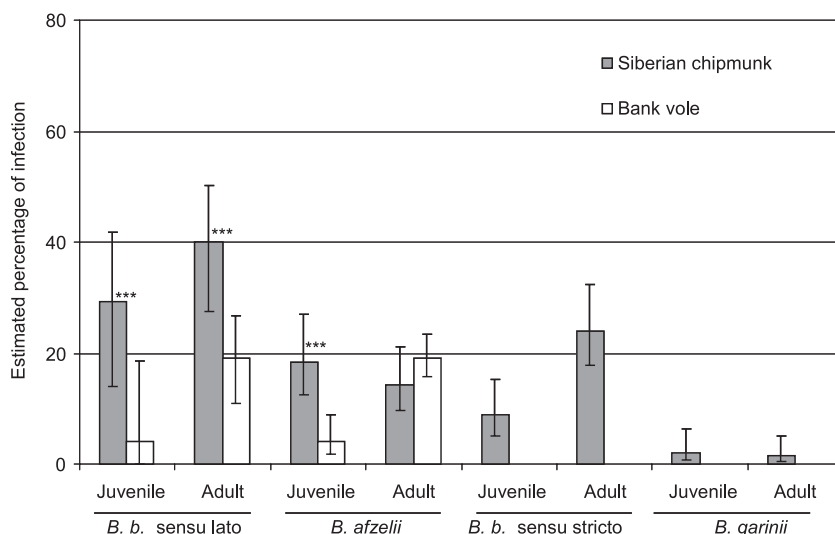


FIG. 2. Estimated infection percentage by *B. burgdorferi* sensu lato genospecies in adult and juvenile rodents. Significant differences ($P < 0.05$, χ^2 test) between Siberian chipmunks and bank voles within age class for *B. burgdorferi* sensu lato and *B. afzelii* are indicated with ***. Errors bars represent the 95% confidence intervals.

where one genospecies, *B. burgdorferi* sensu stricto, predominates (22).

As for diversity, our study showed that chipmunks were found to be infected with all three genospecies that are known to infect rodents (12), all of which also were found in questing ticks in Sénart. *B. afzelii* and *B. burgdorferi* sensu stricto both infected more than 15% of the chipmunks. The reservoir hosts for *B. burgdorferi* sensu stricto are not well known in Europe, having been reported for only a few small rodents (23, 33) and sciurids (17; A. Paulauskas, D. Arnbrasiene, J. Radzijeuskaja, O. Rosef, and J. Turcinaviciene, presented at the 9th International Jena Symposium on Tick-Borne Diseases, Jena, Germany, 15 to 17 March 2007). Therefore, if they are a competent reservoir for *B. burgdorferi* sensu lato, Siberian chipmunks could play an important role in the amplification of this genospecies. Some strains of *B. garinii* (OspA serotype 4) have been isolated in rodents in Europe (14, 16). These subsequent strains were shown to belong to a new genospecies of *B. burgdorferi* sensu lato associated with rodents: *B. bavariensis*. Unfortunately, in this study we were not able to differentiate between *B. garinii* and *B. bavariensis*, so our positive *B. garinii* could be either *B. bavariensis* or a new genotype of *B. garinii* associated with rodents.

To our knowledge, such levels of infection and diversity have rarely been found in a single species at a single location in Europe. Kybicová et al. (24) did find the three rodent-associated *B. burgdorferi* sensu lato genospecies in the muscles of bank voles ($n = 24$) in the Czech Republic. At present, however, there is no evidence that *B. burgdorferi* sensu lato found in muscle can be transmitted to ticks. As ticks tend to attach themselves to ears (34), ear biopsies may be a better means to detect the *B. burgdorferi* sensu lato that can be transmitted to ticks. In Europe, moreover, rates of infection similar to ours have been found for bank voles with the same *B. burgdorferi* sensu lato genospecies (*B. afzelii*) (24; Paulauskas et al., presented). Thus, our study highlights the high prevalence and diversity of *B. burgdorferi* sensu lato genospecies in chipmunks

compared to a common reservoir host of *B. burgdorferi* sensu lato in Europe.

In addition, we found that the prevalence of infection in both chipmunks and bank voles varied yearly and according to age. The temporal variation observed with chipmunks was due to the low infection prevalence of *B. afzelii* in 2006 in adults. The reason for this result is unknown. This low infection prevalence of *B. afzelii* in adults in 2006 further led to a similar infection prevalence in adult and juvenile chipmunks and in a lower infection prevalence of chipmunks compared to bank voles that year. With the exception of this genospecies, adult chipmunks were overall more infected than juveniles. Such an age-related difference in infection prevalence is commonly found in rodents (48). This result suggests that juveniles are less exposed to ticks because of a lower activity (4) and/or that chipmunks develop a cumulative infection by *B. burgdorferi* spp. over time. In other words, once infection is acquired, chipmunks may keep their infection during their lifetime, as do bank voles (18). Infection prevalence in bank voles decreased from 2006 to 2008, which may be due to an increased number of juveniles in the population.

In the case of Siberian chipmunks, two reasons could explain why they were more infected than native rodents and hosted more diverse *B. burgdorferi* sensu lato genospecies: (i) they may harbor more ticks, thereby heightening their exposure, and (ii) they may express little immunity to spirochetes. The two reasons are not exclusive. Compared to bank voles, Siberian chipmunks harbor between 6 and 12 times more ixodid nymphs (J. L. Chapuis, unpublished data), which are the source of infection for reservoir hosts. Because chipmunks are frequently infested by several nymphs (Chapuis, unpublished data), they have a high probability of getting infected, which explains why young chipmunks have a higher prevalence of infection than questing nymphs. In contrast, bank voles, which harbor less than 1 nymph per individual (39), had an infection prevalence similar to that of nymphs for the *B. burgdorferi* sensu lato genospecies that they host (*B. afzelii*). Like the closely related

Tamias striatus in the United States, which is more infested by ticks than the most important reservoir host, *Peromyscus leucopus* (42), chipmunks harbor more ticks than bank voles (39). This could be because they are larger, they use higher vegetation layers, and they may not have acquired the resistance to *I. ricinus* that bank voles have (8). Moreover, chipmunks may be sensitive to *I. ricinus*, as this vector species is not present in Korea, their area of origin (11). However, if we look at *B. afzelii*, which is the only *B. burgdorferi* sensu lato genospecies that infects both species of rodents, chipmunks were only 4 times more infected than voles, yet they were infested with considerably more nymphs (6 to 12 times). Given the extent of their exposure to nymphs, these results suggest that chipmunks are more resistant to this genospecies than voles but are less resistant to other genospecies.

The higher diversity of the *B. burgdorferi* sensu lato genospecies infecting Siberian chipmunks compared to voles could be an intrinsic characteristic of *Tamias* spp. linked either to their Korean origins or to the invasiveness of chipmunks in the Sénart forest. First, there is little evidence in the literature that *Tamias* has a higher tolerance for *B. burgdorferi* sensu lato genospecies than other rodent reservoir hosts. The few studies of Siberian chipmunks that have been conducted in their native area did not find that they were more infected or that they harbored more *B. burgdorferi* sensu lato genospecies than other indigenous rodents (6, 15). However, in North America, the previously cited *T. striatus* (30) is among the most infected rodents after *P. leucopus* (28, 44). Second, Siberian chipmunks may have a higher tolerance for this *B. burgdorferi* sensu lato genospecies because this genospecies is not present in Korea (22), rendering chipmunks naïve. Third, as an invasive species, chipmunks may allocate a greater proportion of resources to growth and reproduction for invasion success to the detriment of an investment in a costly immune response (25).

Three rodent species in the host community of the Sénart forest could influence the dynamics of Lyme borreliosis: chipmunks, bank voles, and wood mice. Wood mice appear to contribute little to the amplification of *B. burgdorferi* sensu lato in the system; they had a low infection prevalence (46) and low density (38). In the present study, we found that introduced Siberian chipmunks had a higher prevalence of *B. burgdorferi* sensu lato and hosted a greater diversity of genospecies, compared to bank voles, while the biomass densities of chipmunks and voles were similar in 2007 and 2008 in our study site (39). If chipmunks are able to transmit the infection back to ticks, i.e., if they are a competent reservoir host for *B. burgdorferi* sensu lato, we hypothesize that there is a high potential for “spillback” from chipmunks to native communities. Under this scenario, the introduced species acquires a native parasite and acts as a new reservoir of infection that increases the infection burden of the native parasite in the native host (20). An alternative hypothesis is that chipmunks are dilution hosts for *B. burgdorferi* sensu lato. This would be the case if chipmunks were “stealing” nymphs away from voles, thereby reducing the probability that voles would acquire infection by *B. burgdorferi* sensu lato, and/or if Siberian chipmunks were less efficient at transmitting *B. burgdorferi* sensu lato to ticks than bank voles. For the moment, no data are available on the reservoir competence of Siberian chipmunks. Finally, since chipmunks are more likely than voles to infect larvae with a higher diversity of

B. burgdorferi sensu lato genospecies, they in fact may be contributing to an amplification of the *B. burgdorferi* sensu lato transmission cycle and may be increasing the risk of Lyme disease transmission to humans.

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